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Characterization techniques and analyses

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Virus detection using nanoparticles and deep neural network–enabled smartphone system

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Detailed protocol

The following information is also available in the published article.

Characterization techniques and analyses

UV-Vis Spectroscopy. Absorption spectra were measured on Beckman Coulter DU800 UV-Vis spectrophotometer. The samples were loaded in 10 mm path length quartz cuvettes and scanned at room temperature.

Transmission Electron Microscopy (TEM). Transmission electron microscopy images were obtained using a JEOL 2100 TEM microscope at an acceleration voltage of 300 kV. The specimens were prepared by dropping 2 µl of the sample onto ultrathin Formvar-coated 200-mesh copper grids and then dried in air. The mean diameter and size distribution histogram of particles were obtained by averaging more than 100 particles from the TEM images using ImageJ software.

Dynamic Light Scattering. Dynamic light scattering (DLS) experiments were performed using Malvern zetasizer (Malvern Instruments, Malvern, UK). The samples were initially filtered through a 0.22 µm filter membrane and allowed to settle overnight for DLS measurements. Ultrapure water (>18 MΩ) from a Pure Lab Ultra water system (ELGA, Ltd) was used as diluent and 3 measurements were recorded for each sample at room temperature. The average values were calculated from the reported results for three different batches.

Fourier transform-infrared (FT-IR) spectroscopy.FTIR spectra in the region of 2000 – 500 cm^{-1} were collected in absorbance mode with a FTS 135 BIO-RAD FT-IR spectrometer.

Enzyme-linked immunosorbent assay (ELISA). We used ELISA to quantify the captured ZIKV on-chip using a sandwich immunoassay kit (RV-403001-ENV-48 ELISA Kit) that was specifically developed to detect ZIKV envelope protein. ZIKV captured on-chip was eluted from the surface of the chip and digested using the sample preparation buffer provided in the kit and the virus concentration was estimated using a standard curve prepared from known virus concentrations. ELISA 96-well plate coated with Anti-Zika ENV Ab was used to capture the target antigen in the samples for 2 h at 37°C. After incubation, the plate was washed 4 times using washing buffer and the plate was dried using paper towels. 100 µl of diluted biotin-Detection Antibody was added and incubated for 60 min. Then, Streptavidin-HRP Conjugate solution (100 µl) was added to each well and incubated for 30 min. The plate was washed 4 times with washing solution after the incubation. 100 µl TMB substrate was added and incubated for 15 min. To stop the reaction, 100 µl of stop solution was added, tapped and mixed gently. When the solution in the wells begins to turn yellow, absorbance of the plate was read at 450 nm using BioTek plate reader.

Agarose gel Electrophoresis. The electrophoretic mobility pattern of the prepared Pt-nanoprobes compared with non-modified PtNPs was evaluated with a horizontal submerged gel electrophoresis apparatus (Mini-SubCell GT, Bio-Rad) using a 0.7% (w/v) agarose gel in TAE buffer (pH 8.5). 10 µl of sample aliquots were loaded into wells. The gel was subjected to a typical voltage of 100 V for 30 min and imaged with a digital camera.

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis.

Protein testing was performed using a Mini-PROTEAN®Tetra System and 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad, Hercules, CA). The samples were digested in sample preparation buffer (2.5 ml of 1 M Tris-HCl pH 6.8, 0.5 ml of ddH_2O, 1.0 g SDS, 0.8 ml of 0.1% Bromophenol Blue, 4 ml of 100% glycerol, and 2 ml of 14.3 M β-mercaptoethanol completed to 10 ml with ddH_2O) and heated for five minutes at 95 ºC on a heat block. Then 15 µl of a protein standard and 20 µl of the samples were loaded on the gel. The voltage was turned up to 90 V and the electrophoresis was continued for 50 min. After electrophoresis was done, the gel was rinsed in water for 3 min and stained in Biosafe Coomassie blue stain for about 1 h. Finally; the gel was de-

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Shafiee, H. (2021). Characterization techniques and analyses. Bio-protocol Preprint. bio-protocol.org/prep945.
2. Draz, M. S., Vasan, A., Muthupandian, A., Kanakasabapathy, M. K., Thirumalaraju, P., Sreeram, A., Krishnakumar, S., Yogesh, V., Lin, W., Yu, X. G., Chung, R. T. and Shafiee, H.(2020). Virus detection using nanoparticles and deep neural network–enabled smartphone system. Science Advances 6(51). DOI: 10.1126/sciadv.abd5354